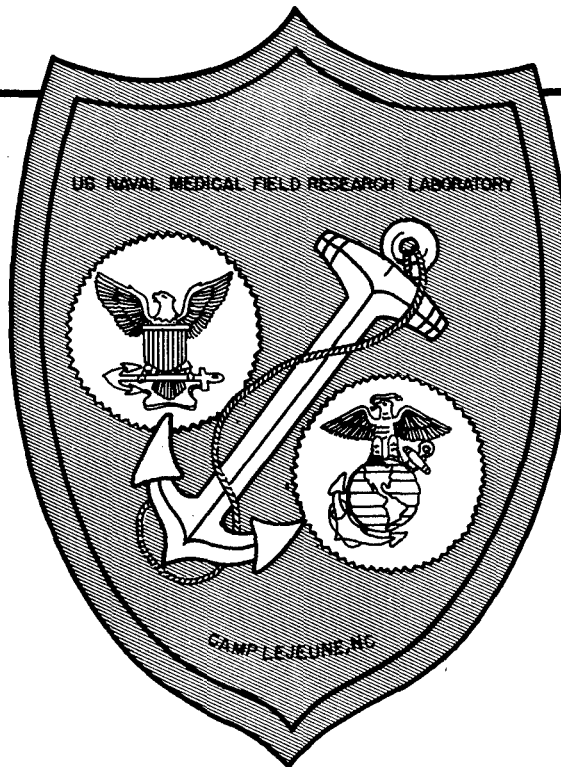


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NONGONOCOCCAL URETHRITIS ASSOCIATED WITH
T STRAINS OF MYCOPLASMA

by

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Bureau of Medicine and Surgery, Navy Department
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SUMMARY PAGE

THE PROBLEM

To review and discuss the current status of T-strain mycoplasma-associated nongonococcal urethritis in the male.

FINDINGS

The development of methods for the isolation and cultivation of T strains of mycoplasma is reviewed. Utilization of some of the important biological properties of T strains for their identification by laboratory methods is discussed, and the detection of T strains of mycoplasma in clinical material by use of a sensitive, specific urease color test medium is suggested.

APPLICATION

Procedures for the laboratory identification of T strains of mycoplasma are presented, and a simple method of detecting their presence in clinical exudates, employing a urease color test medium, is suggested. Our current recommendations for specific treatment of T-strain mycoplasma-associated nongonococcal urethritis are presented. Evidence in support of the infectiousness and possible role of T strains of mycoplasma as one of the major etiologic agents of nongonococcal urethritis in the male is summarized.

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ABSTRACT

The biological properties of T strains of mycoplasma, the development of methods for their isolation and cultivation, their detection in clinical exudates by means of the urease reaction, and their identification by laboratory methods are reviewed. The current status of T-strain mycoplasma-associated nongonococcal urethritis in the male is reviewed and discussed. Evidence in support of the infectiousness and possible role of T strains of mycoplasma as one of the major etiologic agents of nongonococcal urethritis is summarized.

INTRODUCTION

Nongonococcal urethritis (NGU) is a term applied to urethritis from which the gonococcus cannot be demonstrated in smears or in cultures. The disease embraces at least three broad categories of urethritis in man: (1) bacterial urethritis, associated with such genera of bacteria as Proteus, Hemophilus, Mimeae, streptococci and staphylococci, for example; (2) flagellate urethritis, associated with Trichomonas vaginalis; and (3) "abacterial" urethritis, from which no known, well-characterized microorganisms can be demonstrated in smears, or isolated in culture. Generally included under the category of "abacterial" urethritis are two groups of less well-known microorganisms: the Chlamydiae (Bedsonia; TRIC agents), and the Mycoplasma (pleuropneumonia-like organisms; PPLO). Although chlamydial agents have long been known to occur in the human genitourinary tract, their possible role as etiologic agents in nongonococcal urethritis has not been established. Recent refinements in the laboratory techniques for isolation of Chlamydiae from the human genitourinary tract will undoubtedly permit a more accurate assessment of the role of these organisms in nongonococcal urethritis.

MYCOPLASMA-ASSOCIATED NONGONOCOCCAL URETHRITIS

Interest in the mycoplasma as a possible cause of NGU in the male was stimulated by the report by Dienes and Edsall¹ in 1937 of the first recovery of mycoplasma from the human genital tract. The numerous studies reported in the literature since 1937 on the possible role of mycoplasma in the etiology of NGU have, with one exception, all been concerned with the classical (large colony) mycoplasma.²⁻⁸ The possible role of the classical human genital mycoplasma in the etiology of nongonococcal urethritis has been questioned as a result of the nearly equal frequency of these organisms in normal individuals as well as urethritis patients.^{2,3,5,7,8} Further evidence against the classical human genital mycoplasma as causative agents of human nongonococcal urethritis was provided by Willcox,^{9,10} who reported that erythromycin was effective in treating this disease. The classical mycoplasma of the human genitourinary tract, however, were known to be highly resistant to erythromycin. The evidence is therefore convincing that the classical mycoplasma should be regarded primarily as members of the normal genitourinary tract flora, although possibly capable of producing disease under presently undefined circumstances.

NONGONOCOCCAL URETHRITIS ASSOCIATED WITH T STRAINS OF MYCOPLASMA

In our earliest studies of NGU, an attempt was made to determine whether there was any relationship of the then known mycoplasma to this disease

among patients of a venereal disease treatment center. The mycoplasma isolated in primary culture from these patients consisted entirely of the classical, large-colony mycoplasma. The first NGU study group consisted of 38 Negro men with the diagnosis of primary or recurrent nongonococcal urethritis. Classical mycoplasma were recovered from the urethral tract of 20 (53%) of these men. A control group of 215 Negro men from the same medical center population was cultured on the same agar medium and by the same technique. One hundred twenty-one (56%) of these urethritis-free controls yielded classical, large-colony mycoplasma in primary cultures. Thus, in our earliest studies of nongonococcal urethritis, classical mycoplasma were isolated in primary cultures in greater frequency from controls than from NGU patients. It was concluded that the classical mycoplasma were probably unconnected with nongonococcal urethritis and presumably were commensal mycoplasma possibly associated with promiscuous sexual behavior.²

During this same period, however, minute colony-like structures were observed in urethral primary cultures from several patients in the NGU study group. For the better part of a year it was undecided whether these colony-like elements seen in the primary cultures represented a developing agent of some type (possibly a mycoplasma), or whether they were an artifact. For purposes of identification, they were tentatively referred to in our laboratory as "tiny form" or "T form" PPLO colonies. The first published reference to these unusual colonies consisted of a brief description in the legends accompanying two photomicrographs of T colonies in primary agar cultures.² Subsequent studies indicated that these were clearly not artifacts, but a previously undescribed human mycoplasma of quite distinct characteristics and colony morphology from the classical mycoplasma.

It is now known that the culture media employed in those early days were nutritionally and physically inadequate for satisfactory isolation and cultivation of T strains of mycoplasma. The culture medium was a modification of Klieneberger's boiled blood agar medium, but was used without the horse blood treatment.² It was adjusted to a high alkaline pH of 7.6 to 8.0, in the best accepted mycoplasma tradition. The native protein enrichment for this medium consisted of unpooled human ascitic fluid. As one might expect, there were great variations in growth-promoting ability from batch to batch of this enrichment fluid. The Fortner method used for providing a microaerophilic environment in the sealed culture plate was that recommended by Dienes for the cultivation of the classical human mycoplasma.² Subsequent investigations showed that the originally published procedure provided a quite poor gaseous environment for T strains of mycoplasma. One of the greatest difficulties encountered during these early studies of T strains of mycoplasma was the inability to successfully serially propagate T-strain organisms beyond primary isolation. This proved to be a great hindrance to further progress in the study and understanding of these unusual mycoplasma.

DEVELOPMENT OF METHODS FOR CULTIVATION OF T STRAINS OF MYCOPLASMA

Subsequent improvements in culture media and development of methods which led to successful serial cultivation of T strains can be summarized as follows:

1. A change in the native protein enrichment from unpooled human ascitic fluid to human pooled plasma, and then to horse serum, resulted in the first definite and sustained improvement in performance of the culture medium. The first successful serial propagation of T-strain organisms in agar cultures was achieved on media enriched with horse serum in 1962 in our laboratory and Ford's laboratory in Vancouver.¹¹

2. During the same year Ford¹¹ resolved much of the difficulty in serial cultivation of T strains in fluid medium by observing that T strains grew very rapidly in broth cultures and attained maximal titers after only 16 hours incubation at 37°C. Prior to this significant observation, cultivation of T strains in fluid media was unpredictable and generally unsuccessful. It is now known that most of the difficulty resulted from the subculturing of organisms that were well into the logarithmic death phase, which generally occurs after 24 hours incubation at 36-37°C.

3. Also in 1962 a minor but important modification of the Fortner method of microaerophilic incubation was made in our laboratory.¹² This modification consisted of incorporating 1.0% dextrose in the agar medium on which a culture of Serratia marcescens is cultivated in this procedure. Preliminary studies, employing sulfonphthalein indicators in the mycoplasma culture medium, showed that the original Fortner method produced an alkaline reaction in the culture medium. However, if dextrose was incorporated into the medium on which the Serratia culture was grown, an acid reaction developed in the mycoplasma agar medium, and this was accompanied by a simultaneous and significant improvement in performance within the sealed culture plate. The explanation for this improved performance was the increased production of carbon dioxide by Serratia in the presence of a readily available carbohydrate such as dextrose. The modified Fortner technique has now become standard procedure in our laboratory.¹²

4. In 1965 we reported the results of a series of studies on the effect of pH on the cultivation of T strains and classical mycoplasma.¹³ The results of these investigations revealed that the optimal pH for cultivation of T strains was in the acid region of pH 6.0 ± 0.5 . This significant observation proved to be one of the most outstanding contributions to the overall improved performance and reliability of both agar and fluid media for primary isolation and cultivation of T strains.

We are currently recommending a pH 6.0 medium known as A3 medium for the primary isolation and cultivation of T strains of mycoplasma. This medium is prepared from commercially available components and is enriched with sterile unheated horse serum. It is an improved version of the A2 medium described in 1967.¹² A new modification (A5) has recently been developed and is currently being evaluated.

BIOLOGICAL PROPERTIES OF T STRAINS OF MYCOPLASMA

Growth Inhibition by Erythromycin

As mentioned earlier, the human genital classical mycoplasma are known to be highly resistant to the inhibitory action of erythromycin in vitro¹⁴ and in vivo.¹⁵ The finding that nongonococcal urethritis can be effectively treated with erythromycin^{9, 10} provided strong evidence against the classical human genital mycoplasma as causative agents of human nongonococcal urethritis. It is of etiologic significance that T strains of mycoplasma, associated with 60 to 93% of nongonococcal urethritis cases in human males,¹⁶ were found to be sensitive to erythromycin and were selectively inhibited by the drug in vitro.¹⁷ In our laboratory, growth of T strains in an agar system is completely inhibited by 3.12 μ g of the drug per ml at pH 6.0, and 0.78 μ g per ml at pH 7.5.¹⁷ With the exception of the pathogenic Mycoplasma pneumoniae (inhibited by 0.025 to 0.05 μ g of erythromycin per ml),¹⁸ no other human mycoplasma species is known to be sensitive to erythromycin.

Hydrolysis of Urea

The occurrence of a urease enzyme system in T strains of mycoplasma was conclusively demonstrated in our laboratory,^{19, 20} and confirmed by Ford and MacDonald.²¹ Purcell, et al.^{22, 23} have made use of this metabolic capability in the development of a serologic test for antibody to T strains, employing a metabolic inhibition test. T strains are the only known human mycoplasma possessing urease, and this enzyme has proved to be a useful and highly specific biochemical marker.

A sensitive, practical urease color test medium (UCTM) was recently developed in our laboratory and is currently under intensive evaluation.¹⁶ This urease color test medium shows great promise as a diagnostic aid in the detection of T strains of mycoplasma in clinical material (urethral exudate and urine sediment) from patients with nongonococcal urethritis. This clinical test is described more fully in a later section of this report.

Growth Inhibition by Thallium Acetate

Thallium acetate, in addition to erythromycin, is selectively inhibitory to T strains of mycoplasma. This antibacterial agent has been almost universally incorporated in culture media for the cultivation of nearly all known mycoplasma. Growth of T strains is completely inhibited in agar cultures (pH 7.5) by final concentrations of 1:500 thallium acetate.^{12,19} All other classical, large colony mycoplasma (including Mycoplasma pneumoniae) are insensitive to thallium acetate. Freshly isolated T strains are especially sensitive to this agent in primary cultures, and it has been observed that laboratory stock T strains may partially lose their initial sensitivity to thallium acetate. We have recommended the inclusion of penicillin only as the antibacterial agent in culture media for the cultivation of T strains. Mycoplasma media containing thallium acetate should never be employed for the isolation and cultivation of T strains of mycoplasma.

Growth Inhibition by 5-iodo-2'-deoxyuridine (IDU)

This halogenated pyrimidine is known to have antiviral properties and has been used in the treatment of herpes simplex keratitis.²⁴ This compound is also incorporated into the DNA of certain microorganisms by substitution for thymidine, as in the case of vaccinia virus²⁵ and pseudorabies virus.²⁶ Our interest in this halogenated pyrimidine was stimulated by a report that this compound had been employed in the treatment of nongonococcal urethritis, on the assumption that the disease was of virus etiology.²⁷ Prompted by Hutfield's report,²⁷ we investigated the possible inhibitory action of 5-iodo-2'-deoxyuridine against T strains of mycoplasma isolated from patients with nongonococcal urethritis. T-strain organisms were found to be selectively inhibited by IDU, and were completely inhibited by 250 μg of the compound per ml in an agar system of pH 7.6.¹² In contrast, nine classical (large colony) mycoplasma species of human origin (including Mycoplasma pneumoniae) were unaffected by IDU at the highest concentration tested (1,000 $\mu\text{g}/\text{ml}$ at pH 7.6) under the same experimental conditions.¹² Thus, T strains are selectively inhibited in vitro by 5-iodo-2'-deoxyuridine, an antiviral compound successfully employed in the treatment of nongonococcal urethritis. Further, T-strain inhibition by IDU can be reversed by addition of thymidine. These findings suggest that thymidine is incorporated into the DNA of T strains of mycoplasma.

Growth Inhibition by Hydroxyurea

During the course of in vitro tests of compounds that were structurally closely related to urea (a major required metabolite for growth of T strains of

mycoplasma),²⁰ it was observed that hydroxyurea was not utilized as a substrate by T-strain organisms. Instead, it was found that this anti-leukemic compound (hydroxyurea) was found to be selectively inhibitory to growth of T strains of mycoplasma in an agar system of either pH 6 or 7.5. T strains were completely inhibited by a concentration of 500 μ g of hydroxyurea per ml, and some T strains were inhibited by only 100 μ g/ml. Two human classical mycoplasma species examined (Mycoplasma hominis, Type 1, and Mycoplasma salivarium) were unaffected by 500 μ g of hydroxyurea per ml.¹² The fact that growth inhibition of T-strain organisms by hydroxyurea can also be reversed by the addition of thymidine provides additional evidence that thymidine may be incorporated into the DNA of T-strain organisms.

Additional specific biological properties which further characterize T strains of mycoplasma and serve to distinguish T strains from the other human mycoplasma, such as requirements for growth, native protein, amino acids, yeast extract, carbohydrates, and gaseous environment; optimal reaction (pH) for growth; and hemolysis of guinea pig erythrocytes, have been described elsewhere in detail by Shepard.^{12, 28}

SEROLOGIC IDENTIFICATION OF T STRAINS OF MYCOPLASMA

A metabolic inhibition technique for the measurement of antibody to T-strain mycoplasma has been developed^{22, 23} which is based upon the ability of T-strain organisms to metabolize urea with the concomitant production of ammonia, and the ability of specific antiserum to inhibit this ammonia production. Employing this metabolic technique, T strains have been shown to be serologically distinct from the recognized large colony, classical mycoplasma. At least six different serotypes of T strains of mycoplasma have been reported.^{22, 23}

Additional serologic studies of T strains of mycoplasma²⁹ confirm that there are at least six different serotypes of T strains, but that there is variable antigenic overlap between some types, and not all isolates can be typed with the present antisera, and that T strains from patients with Reiter's syndrome are not all of one serotype. Much work remains to be done in working out the techniques and procedures in the serology of T strains of mycoplasma, including the serologic identification of T strains and the detection of antibody in patients with nongonococcal urethritis. There may be a difference between growth inhibiting antibody and antibody which inhibits metabolic reactions (urease activity). T strains of mycoplasma are extremely sensitive to heavy-metal ion contamination and they are sensitive to many compounds (in addition to possible antibody) which are capable of effectively inhibiting growth. Another factor which could confuse the serologic picture is that the organisms may be weakly

antigenic, and that circulating antibodies in the peripheral blood of patients with nongonococcal urethritis are rarely or unpredictably developed. This situation may be comparable to the problem in acute gonorrhea, where classical serologic techniques (complement fixation, for example) are of little value in diagnosis of the disease.

THE OCCURRENCE OF T STRAINS OF MYCOPLASMA IN HUMANS

Isolation of T Strains from Male Nongonococcal Urethritis Patients

The association of T strains of mycoplasma in nongonococcal urethritis in the male has been reported to range from 60 to approximately 80%.²⁹⁻³⁶ The success with which T strains are isolated in primary cultures from NGU patients is a function of two major factors: (1) Performance level of the primary agar or broth culture system employed. Untested substitutions or omissions or other changes in the published formulas of media recommended for isolation and cultivation of T strains of mycoplasma are quite likely to result in considerably reduced isolation rates. (2) Care in the selection of urethritis patients. Patients who are referred for culture studies who report to sick bay only because of fear of having contracted a venereal disease as the result of a recent exposure; patients without urethral discharge and who have only vague symptoms; patients who have been previously treated with broad-spectrum antibiotics and fear that they have not been "cured"; or patients who are treatment failures can be expected in general to yield variable or negative cultures for T strains of mycoplasma. In our laboratory T strains are associated with NGU in approximately 70-84% of the patients we see.

Recently we studied a small special group of 15 NGU patients. These were carefully selected patients who fulfilled rigid criteria for admission to the NGU research study over a one-month period. With one exception, they were NGU patients with previously untreated primary or recurrent nongonococcal urethritis, who had not received treatment for the disease or any other condition with any known drug for 60 days prior to culture. Fourteen (93%) of these 15 NGU patients yielded T strains of mycoplasma in primary cultures. This is the highest rate of association of T strains with nongonococcal urethritis so far reported.¹⁶

Isolation of T Strains from Male Controls

The natural occurrence of T strains of mycoplasma in the genito-urinary tract of men without urethritis ranges from 21% of 100 service recruits in Vancouver³⁴ to 26% of 600 United States Marine recruits at Camp Lejeune,

N. C.¹⁹ The true (corrected) figure is actually 25.8% of 576 men, since 24 Marines were found to have NGU.¹⁶ These Marine recruit controls were cultured in the same manner and on the same culture medium as the NGU study group at Camp Lejeune. Further, the control group consisted of young men of similar age, occupation, and sexual activity as men in the NGU study group. It was interesting to note that the Camp Lejeune controls who admitted recent unprotected sexual intercourse were primarily the men from whom T strains of mycoplasma were isolated in primary cultures. In a recent study of nongonococcal urethritis in London,^{35, 36} T strains were recovered from 13% of 95 healthy men between the ages of 20 and 50 years and no T strains from 15 boys, ages 2 to 12 years. Thus, present evidence indicates that T strains of mycoplasma are associated with nongonococcal urethritis approximately three times as frequently as they are in normal urethritis-free control individuals of similar age, occupation and sexual activity.

Isolation of T Strains from Sites Other Than the Genitourinary Tract

Throat cultures of 71 NGU patients yielded four men (5.6%) who harbored T strains in the pharynx.¹⁶ T strains have also been reported in low numbers from throats of normal individuals.^{23, 29} T strains of mycoplasma have been recovered only once from 70 rectal specimens from 52 male NGU patients and 18 wives of NGU patients. This single rectal isolation was originally identified on the basis of morphology alone, and was classified as an "intermediate" type mycoplasma. On the basis of erythromycin and thallium acetate sensitivity, "intermediate" type mycoplasma colonies have been shown to be T strains of mycoplasma.¹⁷ From this same general group of patients, blood serum from 24 individuals was cultured for T strains. T strains failed to be isolated from the serum of any of five females or 19 male NGU patients, all 24 of whom yielded positive cultures for T strains from the genitourinary tract.¹⁶

Occurrence of T Strains of Mycoplasma in the Female

If T strains of mycoplasma are etiologically associated with venereally transmitted NGU in human males, a certain proportion of female sexual partners should harbor T strains of mycoplasma in the genitourinary tract. Ford and DuVernet³⁴ reported isolating T strains of mycoplasma in vaginal cultures from 44% of 50 females attending a private gynecology clinic in Vancouver. Our earliest study on the occurrence of T strains of mycoplasma in the genitourinary tract of the female was conducted in 1961 in Luzon, Republic of the Philippines. In the town of Olongapo, adjacent to a United States naval station, 2,500 "hostesses" and waitresses in the many bars and clubs in this city reported weekly to the local social hygiene clinic where they were examined for gonococci and gonococcal infection. A sample of 100 of these females was cultured for the presence

of T strains of mycoplasma in the genitourinary tract. These 2,500 "hostesses" comprised approximately 65% of the locally available sexual partners of servicemen at the base, and was the principal female group from which the men were acquiring both gonorrhea and nongonococcal infections at the United States naval station.

Specimens from cervical and urethral sites were cultured separately for T strains of mycoplasma. Of the 100 females cultured, T-strain organisms were recovered from both the cervix and the urethra in 27% of the females, from the cervix only in 11% of the females, and from the urethra only in 23%. Thus, 61% of these females harbored T strains of mycoplasma in the genitourinary tract. From this same group of 100 females, 30% were found to have gonococci on smear or culture; 7% yielded Candida species in culture, 7% had Trichomonas infestations, and 1% had an unidentified Vibrio in culture.³⁷

In a subsequent study of 60 prenatal female military dependents at U. S. Naval Hospital, Camp Lejeune, N. C., T strains of mycoplasma were isolated in primary cultures from the genitourinary tract of 63% of these females.²⁸ It is interesting to note that the clinical findings in these 60 females were essentially negative; that is, there was no evidence of pelvic inflammatory disease, cervicitis, vaginitis, or urethritis in any of the females yielding T strains in primary culture. It is our impression that the female may serve as an asymptomatic carrier of T strains of mycoplasma.

Csonka^{35, 36} reported that the highest proportion of T-strain isolations occurred in women who had marked leucorrhea and who were also sexual partners of men with NGU. Among female control groups, there was a considerable range in the distribution of T strains in the urine. Isolation rate was highest in pregnant women, followed by non-pregnant adults, most of whom were unmarried, then post-menopausal women (with a low incidence), and finally girls between the age of 13 and 18 years presumed to be virgins from whom no T strains were isolated.^{35, 36}

In a continuing study of wives of military men with nongonococcal urethritis at Camp Lejeune, T strains of mycoplasma are generally isolated from the wife as well as the husband. Effective and lasting eradication of NGU in the husband in most cases is accomplished only by simultaneous treatment of both the husband and the wife, and achievement of negative cultures for T strains of mycoplasma in both marital partners. In the case of the married NGU husband, it has been our experience that failure to include the wife in the overall picture is likely to result in failure to achieve lasting cure in the husband. In such cases the husband is re-treated repeatedly, only to become re-infected from his T-strain positive wife. This "ping-pong" type of infection, treatment, and re-infection of the husband can only be broken by simultaneous treatment of both marital partners, as emphasized above.

The Association of T Strains of Mycoplasma with Human Reproductive Failure

In 1967, an unusual type of mycoplasma was isolated from the chorion, decidua, and amnion of a female patient who experienced a spontaneous middle trimester abortion.^{38, 39} Histologically, the decidua showed extensive necrosis and subacute inflammation; the fetal membranes and umbilical cord vessels were severely inflamed, and infection of the membrane appeared to be of long duration. Unusual sclerosis of the placental villi was also observed. Acute inflammatory exudate filled the lumens of the bronchi and stomach. There was no evidence of subjacent tissue reaction. The patient, a 27-year-old woman in generally good health, whose first four pregnancies were uneventful, had expelled the female fetus five days after spontaneous rupture of the membranes in the seventeenth week of gestation. There was no fever or other systemic evidence of infection. The mycoplasma isolated from the fetal membranes of this patient was identified in this laboratory (NMFRL) as a T strain of mycoplasma,^{16, 38, 39} and the name "Boston" T strain was suggested for this mycoplasma until further investigations could be made to determine its appropriate taxonomic status. It is interesting to note that the 27-year-old female patient and her husband were both examined culturally for mycoplasma following the abortion. The "Boston" T strain was reisolated from the cervical swab and urine sediments of the wife and from a urine specimen from the husband. Cultures of endometrial biopsy, the husband's urethra, and the throats of husband and wife were negative for mycoplasma.

Subsequently, the "Boston" T strain was recovered from membranes of three of six spontaneous abortions or premature births and from cervical cultures of five of ten women with a past history of repeated spontaneous abortions. It is of interest that this "Boston" T strain, identified in our laboratory as a member of the T-strain group, proved to be extremely fastidious initially in its growth requirements, or sensitive to presently unknown inhibitory substances, and grew very slowly and with difficulty in agar culture. Following continuous serial subculture in fluid medium and on agar medium, however, the organism proved to be a typical T strain and was morphologically identical and similar in cultural behavior to T-strain organisms isolated from NGU patients. The mycoplasma was urease positive. It is significant that 10 different combinations of agar medium in our laboratory were completely negative for mycoplasma on primary isolation attempt, and only one of nine different combinations of fluid medium was capable of growing out this extremely fastidious "Boston" T strain in primary isolation. Of the three frozen tissues submitted to us for study (amnion, chorion, and decidua), the "Boston" T strain was recovered from the chorion only. Further, successful recovery was accomplished only in a urease color test medium which gave a positive urease reaction after five days of continuous incubation at 36°C. This observation emphasizes the importance of the urease color test medium, not only for routine diagnostic work with T strains, but as a primary isolation medium under special conditions.¹⁶ The suggestion that

T strains of mycoplasma may play a role in human reproductive failure in the female is a new concept and it opens up a new and unexplored field of investigation.

TREATMENT OF NONGONOCOCCAL URETHRITIS IN THE MALE

In our early studies of nongonococcal urethritis we recommended treating the disease with oxytetracycline or tetracycline orally, 500 mg every 6 hours for 4 to 7 days.¹⁶ The drugs were generally administered over a 4-day period, and a cure rate of approximately 50% was obtained. In view of this low cure rate, the drug (either oxytetracycline or tetracycline) was administered for 5 to 7 days. Finally, a 9- to 10-day course of treatment with oxytetracycline or tetracycline achieved a cure rate in the neighborhood of 85% with a single course of treatment.³² One possible explanation for the low cure rate achieved with the tetracycline group over a period of 4 days is that it generally requires about 4 days of continuous treatment (500 mg q.i.d.) for the urethral discharge to cease in the average patient. Our current recommendations for primary treatment of nongonococcal urethritis in the male, employing either oral oxytetracycline or tetracycline, is 500 mg q.i.d. for 10 days.

The discovery that T strains of mycoplasma (in contrast to the classical, large colony human genital mycoplasma) are selectively inhibited by erythromycin in vitro¹⁷ added a new antibiotic agent to the limited armamentarium of drugs useful in the treatment of NGU infections in the male. Our current recommendation for treatment of NGU with erythromycin is 500 mg q.i.d. for 10 days with mild alkalization with sodium bicarbonate. The latter adjunctive therapy is suggested in view of the greater activity of erythromycin in a slightly alkaline environment, and the fact that such an environment is less favorable to growth of T strains of mycoplasma. Erythromycin has also proved to be an excellent re-treatment drug for NGU cases failing treatment with tetracyclines. Our laboratory studies of new and as yet untried chemotherapeutic agents which may have usefulness in the treatment of NGU infections is continuing.

IDENTIFICATION OF T STRAINS OF MYCOPLASMA

In earlier studies of T-strain mycoplasma-associated nongonococcal urethritis, identification of these organisms was based only upon the highly characteristic size, morphology and staining reaction of agar colonies of T strains in primary cultures or in cultivation.^{12,40} During the period of development of methods (special techniques, and appropriate culture media for the primary isolation and cultivation of T strains of mycoplasma), biochemical studies provided new knowledge and understanding of the unusual metabolism of

T-strain organisms. It is now possible to utilize certain biochemical procedures for the identification of T strains of mycoplasma and their differentiation from other members of the human classical mycoplasma group.^{12, 19, 28}

Some of the more important biological characters which can be employed for the detection of T strains of mycoplasma in clinical material and their identification in culture are briefly summarized as follows:

1. Optimal reaction for growth: pH 6.0 ± 0.5 .
2. Gaseous environment: Microaerophilic, poor growth aerobically; no growth anerobically; require carbon dioxide for best growth (10-15%).
3. Carbohydrates: The usual carbohydrates are not fermented.
4. Urea: Actively hydrolyzed with the accumulation of ammonia. T strains are the only known human mycoplasma possessing an active urease.
5. Hemolysis of erythrocytes: Guinea pig erythrocytes are hemolyzed in the red cell agar overlay technique. (However, the culture medium must be freshly prepared.) The hemolytic plaques are very small, and the procedure is not recommended as a routine procedure.
6. Thallium acetate sensitivity: T strains are sensitive to 1:500 thallium acetate in an agar system of pH 7.5. T strains are the only known mycoplasma sensitive to thallium acetate.
7. Erythromycin sensitivity: T strains are selectively inhibited by 10-25 $\mu\text{g/ml}$ of erythromycin in an agar system of pH 7.5.
8. 5-iodo-2'-deoxyuridine (IDU) sensitivity: T strains are selectively inhibited by 125 $\mu\text{g/ml}$ of 5-iodo-2'-deoxyuridine in an agar system of pH 6.
9. Hydroxyurea sensitivity: T strains are selectively inhibited by 500 $\mu\text{g/ml}$ of hydroxyurea in an agar system of either pH 6 or 7.5.
10. Growth inhibition by specific antiserum: Inhibition can be demonstrated by the use of specific antiserum, employing the filter paper disk method in an agar culture system or by employing the metabolite inhibition test. The serologic relationships and techniques in general have not been fully explored, and much work remains to be done in this field.

Urease Color Test Medium

The urease reaction has proved to be a highly useful biochemical test for the detection of T strains of mycoplasma in clinical material, and for the identification and differentiation of T strains from other human mycoplasma. A urease color test medium (U9) is currently under intensive evaluation in our laboratory.⁴¹ This medium is of high sensitivity and specificity for T strains of mycoplasma, and it is relatively free of characteristics which tend to result in false positive reactions. The agreement between positive urease reactions in the urease color test medium and primary isolations of T strains in agar cultures is essentially 100%. During the current evaluation, the presence of T strains of mycoplasma in urethral exudate from NGU patients was detected in two instances by the urease color test medium in the face of negative primary agar cultures of the same exudates. The tubes of urease color test medium required an extended incubation period of 4 and 8 days, respectively, before the urease reactions became positive. In both cases, however, T-strain organisms were recovered in agar subcultures from the urease color test broths at the time the urease reactions became positive.⁴¹ The urease color test medium, in its present U9 formulation, should prove to be a valuable diagnostic aid to the clinician for the detection of T strains of mycoplasma in urethral discharges of patients with nongonococcal urethritis and from genitourinary tract specimens from their female consorts or wives. Use of this special medium may be of particular advantage in those instances where specialized agar cultures for primary isolation of T strains of mycoplasma may be impractical or impossible. The U9 Urease Color Test Medium is currently undergoing preliminary field testing and evaluation aboard one of the naval ships of the Sixth Fleet on training maneuvers in the Mediterranean and at the Base Dispensary, U. S. Naval Station, Guantanamo Bay, Cuba.

T STRAINS OF MYCOPLASMA IN NONGONOCOCCAL URETHRITIS

The present evidence which suggests that T strains of mycoplasma may be one of the etiologic agents of nongonococcal urethritis in humans is summarized briefly as follows:

1. T strains of mycoplasma are associated in 60-93% of male nongonococcal urethritis patients. In contrast, T-strain organisms have been isolated from only 21-26% of urethritis-free, normal controls. The controls were cultured by the same technique and on the same culture medium as those men in the NGU study group. Further, the controls were of similar age, occupation, and sexual activity as men in the NGU study group. Thus, T strains of mycoplasma are associated approximately three times as frequently in NGU patients as in normal, urethritis-free controls.

2. In T-strain-associated NGU patients who are successfully treated (with tetracyclines or with erythromycin), T-strain organisms disappear from the genitourinary tract and can no longer be isolated in post-treatment follow-up cultures for mycoplasma, and patients become and remain free of symptoms.

3. T-strain-associated NGU patients who have been unsuccessfully treated (with tetracyclines or erythromycin) (a) continue to have urethral discharge in spite of treatment, and continue to yield T-strain organisms in primary cultures; or (b) become temporarily asymptomatic but continue to yield T strains of mycoplasma in post-treatment cultures. The latter patients can generally be predicted to experience a return of urethritis (relapse to treatment) in from 7 to 21 days or more. In our experience, ultimate successful re-treatment is indicated both by clinical cure and by elimination of T-strain organisms from the genitourinary tract, as judged by carefully performed post-treatment follow-up cultures.

4. Present evidence suggests that married T-strain-associated male NGU patients who are successfully treated may become re-infected from their wives (if their wives are carriers of T-strain organisms). Successful treatment of NGU in such husbands appears to depend upon successful elimination of T-strain organisms from both marital partners.

5. T strains of mycoplasma are inhibited in vitro by the same drugs which are effective in treatment of nongonococcal urethritis in humans. These drugs are oxytetracycline, tetracycline, erythromycin, and 5-iodo-2'-deoxyuridine. The latter drug was used in the treatment of nongonococcal urethritis in the belief that the disease was of viral origin.²⁷

Thus, the close association of T strains of mycoplasma with nongonococcal urethritis, their intimate association with the clinical course of the disease, and their response following treatment of the disease, suggest that T-strain organisms play an important role in the etiology of the disease. Evidence accumulated to date strongly supports their role as one of the major causative agents of nongonococcal urethritis in the human male.

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